

EXHIBIT RAS-11

This is exhibit RAS-11 referred to in Declaration Under 37 C.F.R. 1.132 by Richard Anthony Strugnell dated 27.9.01

  
Richard Strugnell

## EPITOPE MAPPING OF ANTI-BREAST AND ANTI-OVARIAN MUCIN MONOCLONAL ANTIBODIES

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**Abstract**—Anti-breast cancer antibodies (BC2, HMPV and 4B6) and an anti-ovarian cancer antibody (OM1) were found to react with mucins—indeed with the protein core encoded by the MUC1 gene. This gene contains a VNTR (variable number of tandem repeats) encoding a 60 bp (=20 amino acids) repeat sequence and within this amino acid sequence SAPDTRPAP was predicted, by hydrophilicity analysis, to be the immunogenic peptide sequence. The four antibodies were shown to react with MUC1 VNTR encoded peptides in direct binding and inhibition studies. The precise reactivity of the 4 mAbs was mapped using ELISA in both solid and liquid phase, and demonstrated the epitopes to be: APDTR (BC2 and HMPV), PDTR (4B6) and DTRPA (OM1). By using the pepscan method, the epitopes were shorter (PDTR, DTR and DTRP). However when these short peptides (except DTR) were synthesized they did not react; flanking amino acids are needed for the epitopes. Clearly several different methods should be used to define the reactive epitope. Within (S)APDTR, major amino acid substitutions could be made—even of three to four amino acids without altering antibody binding, provided that P and R were not substituted. It was of interest that an anti-ovarian cancer antibody gave similar anti-peptide reactions to the anti-breast cancer antibodies; apparently MUC1 peptides in ovarian cancer are the same as in breast cancer.

### INTRODUCTION

Mucins are highly expressed in many tumours—particularly carcinomas of the breast, colon, pancreas and ovary, and as a result many monoclonal antibodies raised against these carcinomas react with mucins (McKenzie and Xing, 1990). Of recent interest has been the cloning of cDNAs coding for the protein core of four of these mucins: MUC1 is expressed in a number of different tissues and cancers of breast, pancreas and ovary (Gendler *et al.*, 1987; Siddiqui *et al.*, 1988; Lan *et al.*, 1990); MUC2 and 3 are expressed in intestinal mucins (Gum *et al.*, 1989; 1990); MUC4 in tracheo-bronchial mucin (Prochet *et al.*, 1991). It has been found that monoclonal antibodies reacting with the MUC1 mucin can react either with carbohydrate or peptides—the sequence of the latter containing a variable number (40–80) of tandem repeats (VNTR) giving rise to the sequence GSTAPPAHGVTSAPDTRPAP (Xing *et al.*, 1989a; Wreschner *et al.*, 1990; Lightenberg *et al.*, 1990; de Kretser *et al.*, 1985; Gendler *et al.*, 1988). We have recently mapped the epitopes of some anti-breast cancer mucin antibodies to the 5 amino acid peptide APDTR within the VNTR of MUC1 (Gum *et al.*; Xing *et al.*, 1990). We now report on the epitope mapping of anti-mucin antibodies, OM1, raised against ovarian cancer cell line (de Kretser *et al.*, 1985); 4B6 raised against

mammary mucin (HMFG) and HMPV raised against HMFG but screened against mammary mucin core protein peptide p1-24 (PDTRPAPGSTAPPAHGVTSAPDTR); and the results were compared with the previously described anti-APDTR BC2 antibody (Xing *et al.*, 1990, 1991). By using different techniques, it was shown that the reactive epitopes were APDTR (BC2 and HMPV), the PDTR (4B6) and DTRPA (OM1) although using the pepscan method, shorter epitopes were defined.

### MATERIALS AND METHODS

#### Monoclonal antibodies

Four mAbs BC2, 4B6, HMPV, OM1 were used in the study (Xing *et al.*, 1989b; de Kretser *et al.*, 1985). For BC2 (IgG1) and 4B6 (IgM) human milk fat globule membrane preparation (HMFG) was the immunogen; HMPV (IgG1) was prepared by immunization of BALB/c mice with HMFG and the spleen cells boosted *in vitro* with the peptide p1-24 (see below) (Boss *et al.*, 1984; Xing *et al.*, 1989b). OM1 antibody (IgM) was made against an ovarian cancer cell line (de Kretser *et al.*, 1985, 1988; Layton *et al.*, 1990), and was obtained from Medical Innovations Ltd, Labrador, Queensland. Previous studies showed that BC2 defined a high molecular weight glycoprotein (product of the MUC1 gene) and reacted with the peptide APDTR (Xing *et al.*, 1990). Mab 5C1 (IgM) to colon cancer mucin (Teh *et al.*, 1990) was used as a negative control.

#### Peptide synthesis

Peptides whose sequence was derived from the VNTR of mammary mucin were made with an Applied

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**Abbreviations:** BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; HMFG, human milk fat globule membranes; mAb, monoclonal antibody; PVC, polyvinylchloride; VNTR, variable number of tandem repeats.

Table 1. Reaction of mAbs with synthetic peptides tested by ELISA<sup>a</sup>

| Peptide <sup>b</sup> | Amino acid sequence         | BC2             | HMPV | 4B6 | OM1 | 5C1 |
|----------------------|-----------------------------|-----------------|------|-----|-----|-----|
| p1-24                | PDTRPAPGSTAPPAHGVTSAPDTR    | ++ <sup>a</sup> | ++   | ++  | +   | -   |
| p1-15                | PDTRPAPGSTAPPAH             | -               | -    | -   | +   | -   |
| A-p1-15              | APDTRPAPGSTAPPAH            | +++             | ++   | ++  | +   | -   |
| TSA-p1-24            | TSAPDTRPAPGSTAPPAHGVTSAPDTR | +++             | +++  | +++ | +   | -   |
| p13-32               | PAHGVTSAPDTRPAPGSTAP        | +++             | ++   | +   | +   | -   |
| T4N1 <sup>c</sup>    | KTLVLGKEQESAEPLCECY         | -               | -    | -   | -   | -   |
| HMFG <sup>d</sup>    |                             | +++             | +++  | +++ | ++  | -   |

<sup>a</sup>Direct binding ELISA absorbance at 405 nm; (-) ≤ 0.25; 0.25 < (+) ≤ 1.0; 1.0 < (++) ≤ 1.5; 1.5 < (+++) at a mAb concn of 40 µg/ml.

<sup>b</sup>In the peptides the numbers refer to the 24 amino acid sequence with p1-24 (Xing *et al.*, 1991) as the standard.

<sup>c</sup>T4N1 is N-terminal of mouse CD4, and was used as negative control peptide.

<sup>d</sup>For comparison HMFG was also prepared and coated to plates at 10 µg/ml and tested in an ELISA.

Biosystems 430A automated peptide synthesizer (Foster City, CA, U.S.A.) (Xing *et al.*, 1989a; Geysen *et al.*, 1987) (Table 1). In addition, three different groups of peptides were synthesized on polyethylene pins (Cambridge Research Biochemicals, Cambridge, U.K.) ("pepscan" method) (Xing *et al.*, 1990, 1991; Geysen *et al.*, 1987). The first group consisted of twenty overlapping 6-mer peptides, e.g. PDTRPA, DTRPAP, TRPAPG, . . . . APDTRP which were made to map the epitope of mAbs (Fig. 2). To evaluate the role of each amino acid in the peptide recognized by mAbs, a second group 120-6-mer peptides were made by changing one amino acid at each site in the parent peptide SAPDTR (Fig. 5) (Xing *et al.*, 1991). In the third group 29-6-mer peptides were synthesized; they were designed depending on the "substitution permissible" amino acids S, A, D and T in the BC2 and HMPV epitopes (S)APDTR (Xing *et al.*, 1991). [In earlier studies (Xing *et al.*, 1990) it was

found that in SAPDTR, neither P nor R could be substituted with any other amino acid—the remaining amino acids S, A, D and T are referred to as being "permissible"]. Each 6-mer peptide in this group contained P and R, and up to 3 or 4 amino acids were substituted on the pins; their activity was tested with the 4 mAbs (Fig. 6).

### ELISA

Direct binding ELISAs and inhibition assays in liquid phase were performed as described (Xing *et al.*, 1990). In addition, some short peptides were coupled to BSA and tested in direct binding ELISAs, since short peptides (less than 15 amino acids) do not readily bind to PVC plates (Briand *et al.*, 1985; Xing *et al.*, 1990). Some peptides (more than 15 amino acids) such as p1-15, A-p1-15, p5-20 and P1-24 were also coupled to BSA for

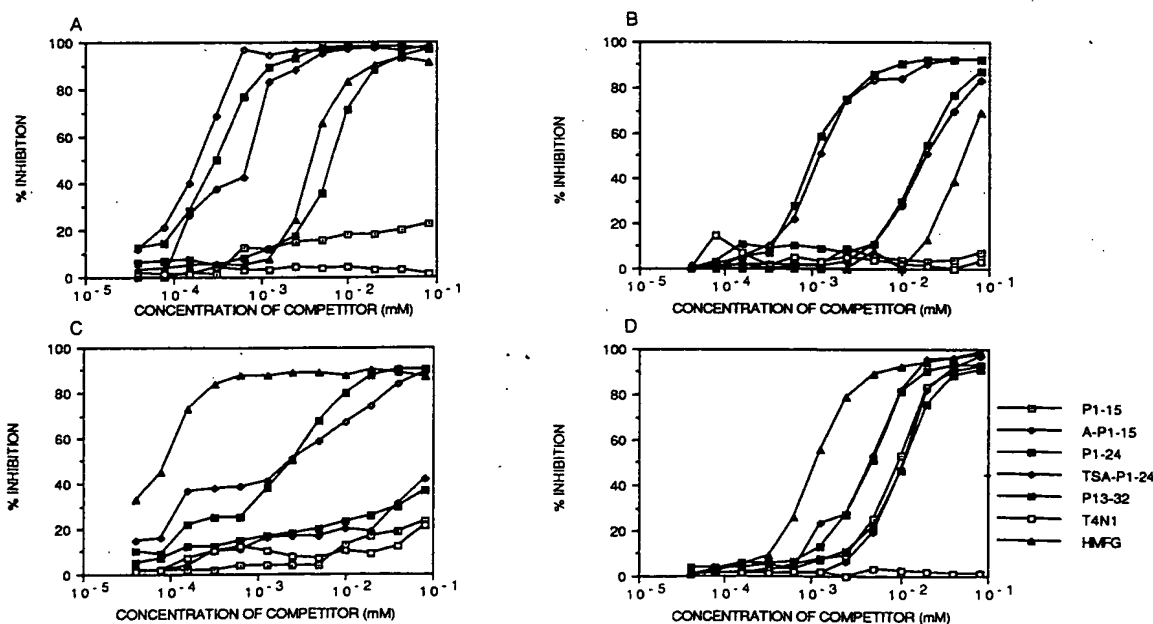


Fig. 1. Percentage inhibition of the binding of the mAbs BC2 (A), HMPV (B), 4B6 (C) and OM1 (D) to HMFG by synthetic MUC1 peptides, negative control peptide T4N1 and HMFG at increasing concns ( $0.4 \times 10^{-4}$ –0.08 mM) in an ELISA assay.

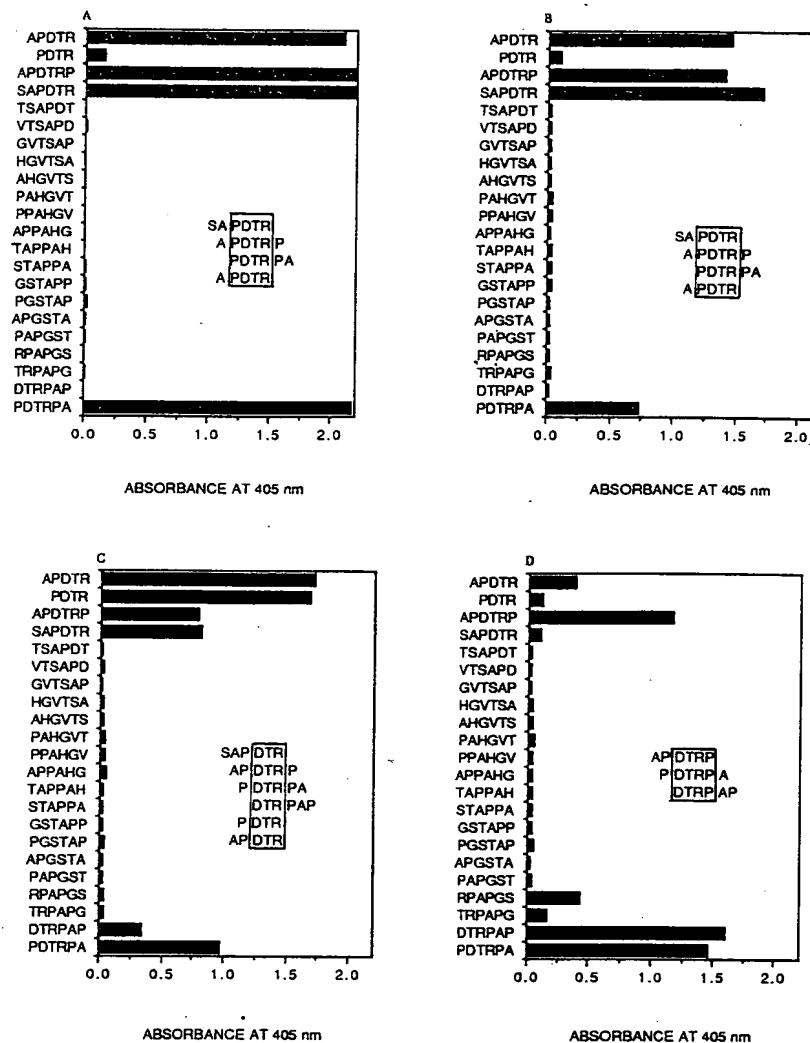


Fig. 2. Binding of mAb BC2 (2  $\mu$ g/ml) (A), HMPV (2  $\mu$ g/ml) (B), 4B6 (8  $\mu$ g/ml) (C) and OM1 (2  $\mu$ g/ml) (D) to 20 6-mer overlapping peptides derived from the 20 amino acid tandem repeat in ELISA assay. Four-mer PDTR and 5-mer APDTR were also tested by ELISA; height of black bar equals absorbance value. The common amino acids recognized by mAbs are shown in the boxes.

comparison with short peptide conjugates. For testing peptides on the pins, the ELISA technique was used with minor modification (Xing *et al.*, 1990).

## RESULTS

### Reaction of mucin antibodies with HMFG and with synthetic peptides

In direct ELISA and inhibition tests all four antibodies reacted not only with native mucin (HMFG) but also with some of the synthetic peptides but not with a control peptide T4N1 (Table 1, Fig. 1). There were some similarities in the reactions (summarized in Table 1); for example none of the antibodies except OM1, reacted with the peptide p1-15; all were reactive with A-p1-15; and strong reactions were noted with the peptides p1-24, p13-32 and TSA-p1-24. The results with the antibodies 4B6, HMPV are similar to those previously reported for BC2 (Xing *et al.*, 1990, 1991), indicating that the epitopes for all these antibodies were similar (although not iden-

tical) and were likely to be within the amino acids SAPDTRPAP, the hydrophilic region in the VNTR shown by a Hopp and Woods analysis (Hopp and Woods, 1983). The results obtained from direct binding to peptides (Table 1), were confirmed by inhibition studies with the peptides in liquid phase (Fig. 1). These three antibodies reacted strongly with TSA-p1-24—a peptide of interest as it contains 2 SAPDTR epitopes compared to one in p1-24. It was noted that OM1 was different to the other antibodies in that it bound weakly to p1-15, and the p1-15 peptide gave the same degree of inhibition as the whole p1-24 peptide (Table 1, Fig. 1D). However, OM1 reacted more strongly with A-p1-15 than with p1-15 in the direct binding assay (Table 1). From these studies it appeared that the new antibodies HMPV and 4B6 reacted with peptides similarly to BC2 (Xing *et al.*, 1991). The studies were subsequently extended to delineate the epitopes involved using the pepscan method and to establish which amino acid substitutions (from 1 to 4 amino acids) are compatible with retention of antigenic activity.

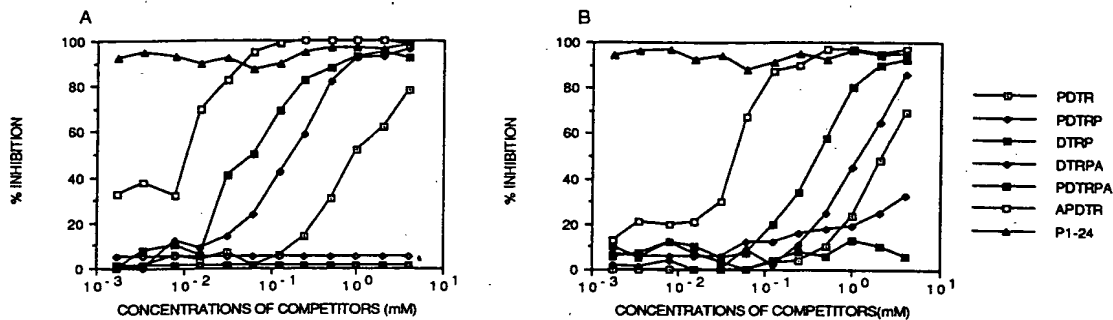


Fig. 3. Percentage inhibition of binding of the mAbs BC2 (25 ng/ml) (A), and HMPV (50 ng/ml) (B) to HMFG by short peptides (4–6 mer) and p1-24 at increasing concns ( $2 \times 10^{-3}$ –4 mM). Results with mAbs 4B6 and OM1 are not shown as no inhibition occurred.

#### Determination of peptide epitopes reacting with the antibodies

The synthesis of 6-mer overlapping peptides on polyethylene pins led to the mapping of the linear epitopes recognised by the four antibodies. Reactions of the antibodies on 20–6-mer peptides, the 4-mer PDTR and 5-mer APDTR synthesized on the pins are shown (Fig. 2), where all antibodies gave similar but not identical results. It was noted that PDTR was non-reactive with BC2, HMPV and OM1 while PDTR was reactive with 4B6. Also shown is the minimum epitope reactive with antibody, derived by comparing the common sequence present in the reactive peptides (Fig. 2). By this method, the epitope for BC2 and HMPV antibodies is PDTR; for 4B6 it is DTR and for OM1 it is DTRP (Fig. 2, Table 2). Thus, although the antibodies recognized similar epitopes, different reactions were noted. However previous work with BC2 (Xing *et al.*,

1990, 1991) and that described above for HMPV had indicated that APDTR was the reactive epitope and not PDTR (Fig. 1) (Xing *et al.*, 1991). For example, the peptide p1-15, containing PDTR, was clearly non-reactive with BC2, 4B6 and HMPV in direct binding and inhibition assays, whereas A-p1-15, containing APDTR was reactive (Xing *et al.*, 1990). However, when examined using the "pepscan", PDTR was the common epitope (Fig. 2, Table 2).

To further examine the differences noted, three methods were used: inhibition ELISA with the peptides in liquid phase; the Pepsan method and a direct binding ELISA including using some short (less than 6 amino acids) and long peptides (more than 15 amino acids) coupled to BSA. First, inhibition studies were performed using the short peptides to determine the correlation between the "Pepsan" method and inhibition studies of epitopes (Fig. 3). The studies performed with BC2 and HMPV indicated that PDTR at higher concns ( $> 1$  mM)

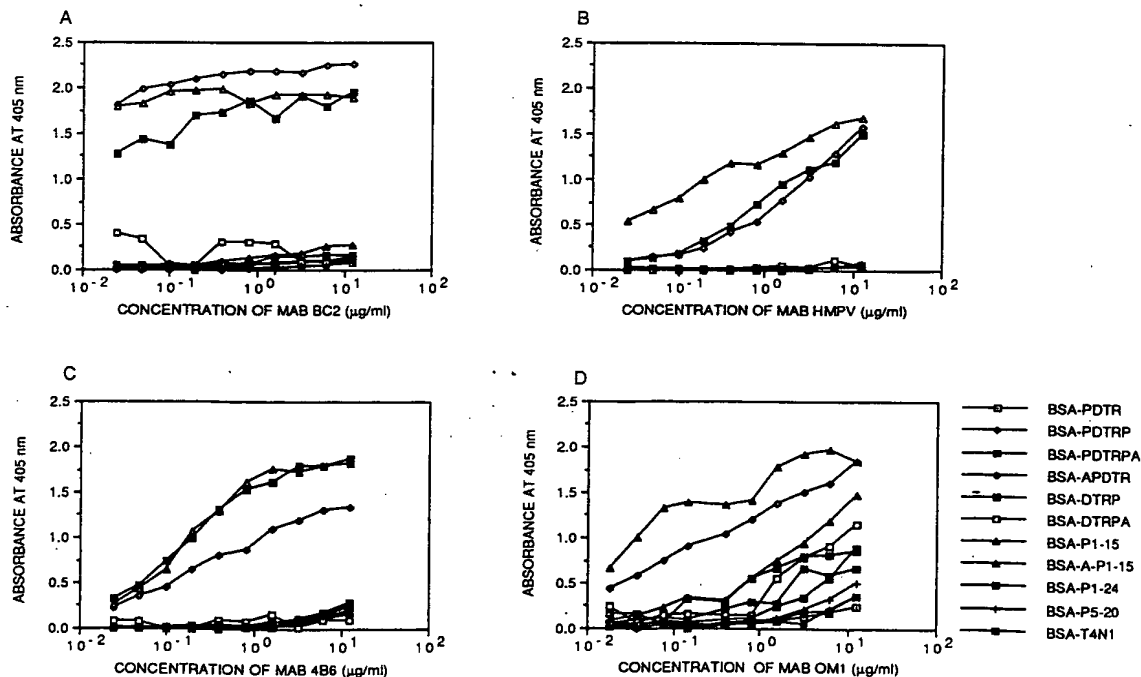


Fig. 4. Binding of mAbs BC2 (A), HMPV (B), 4B6 (C) and OM1 (D) to BSA-peptide conjugates at increasing concns (0.024–12.5  $\mu$ g/ml) in an ELISA assay.

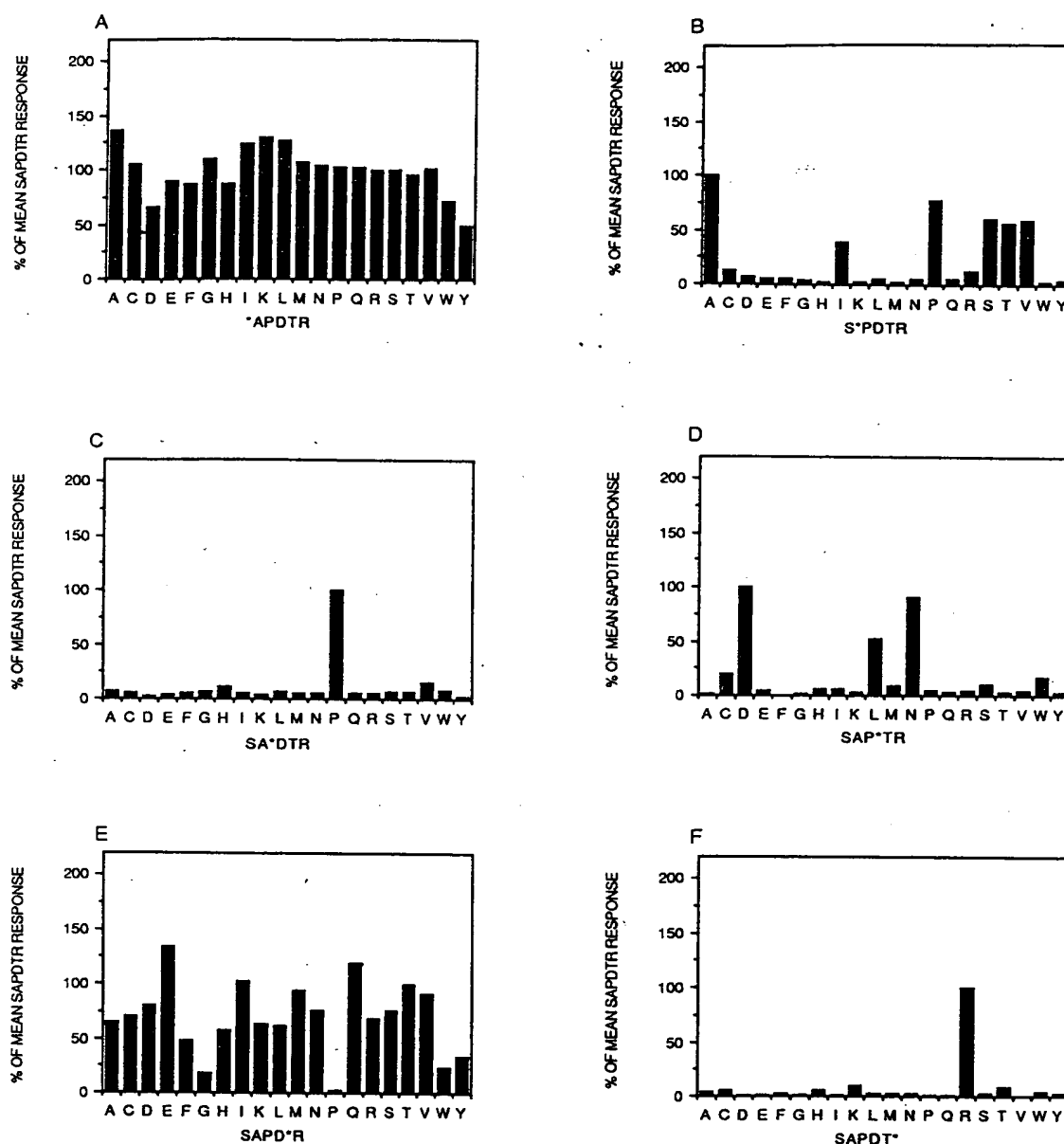


Fig. 5. Replacement set analysis of SAPDTR recognized by mAb HMPV [BC2 results shown elsewhere (Xing *et al.*, 1991)]. The percentage in each block (A-F) represents the reaction of mAbs with SAPDTR peptides containing the single amino acid substitution marked by an asterisk. ELISA values for the six replicates of the parent sequence SAPDTR were averaged and taken as 100% for comparison with the values obtained with the replacement analogs.

could indeed give some degree of inhibition but not as strongly as PDTRP or PDTRPA (Fig. 3B). However, the best inhibition was obtained with the small peptide APDTR which might be the minimum epitope of HMPV and BC2 (Fig. 3A). It appeared that the flanking amino acids (P at C-terminal and A at N-terminal) to PDTR improved the reaction of the antibodies BC2 and HMPV with PDTR itself (Fig. 3). By contrast, none of short peptides inhibited 4B6 and OM1 binding to HMFG (data not shown). Secondly, using the "Pepscan" method the 4-mer peptide PDTR, and 5-mer APDTR were made on the pins and tested with the 4 mAbs (Fig. 2). The 4-mer PDTR did not bind HMPV, BC2 or

OM1, but reacted with 4B6, indicating this to be the 4B6 epitope and distinguishing it from the other 3 antibodies. Thirdly, the small peptides were coupled to BSA and tested in a direct binding ELISA (Fig. 4): HMPV and BC2 bound to BSA-p1-24, BSA-APDTR, BSA-A-p1-15, but not to BSA-PDTR, BSA-P1-15 and BSA-DTRP, BSA-PDTRP, BSA-PDTRPA and BSA-p5-20, which did not contain APDTR (Table 3), confirming the reactivity with APDTR and not PDTR. OM1 bound to BSA-p1-15, BSA-A-p1-15, BSA-DTRPA, BSA-APDTR and weakly with BSA-DTRP; it did not react with BSA-PDTR and BSA-PDTRP. Surprisingly BSA-PDTRPA, which contained the

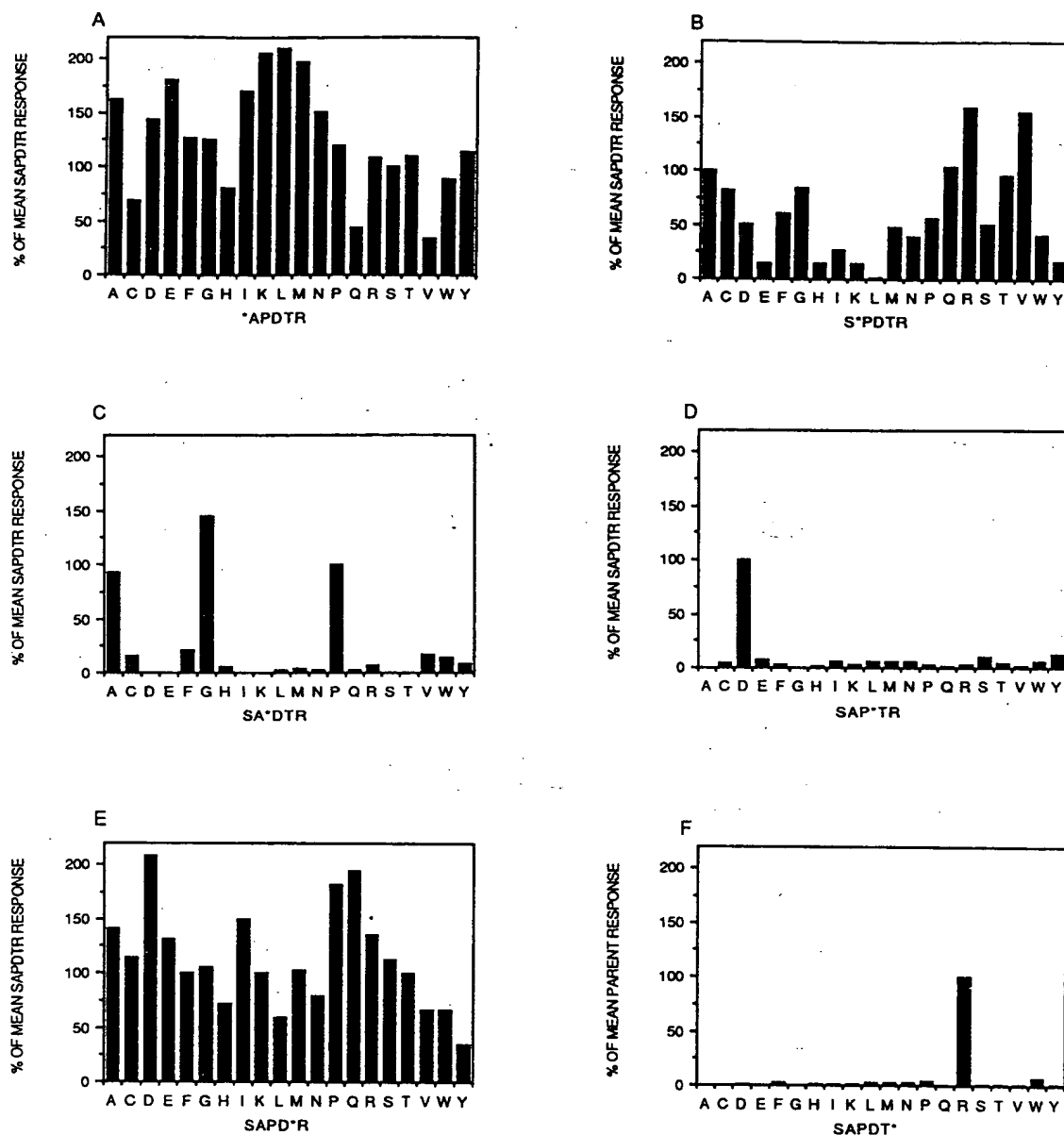


Fig. 6. Replacement set analysis of SAPDTR recognized by mAb 4B6 (see legend Fig. 6 for further detail).

OM1 epitope DTRPA, was non-reactive with OM1 (Fig. 4D, Table 3). 4B6 was only reactive with BSA-APDTR, BSA-pl-24 and BSA-A-pl-15, and not with BSA-pl-15 or other peptide conjugates (BSA-PDTR, BSA-DTRPA, BSA-DTRP, BSA-PDTRP and BSA-PDTRPA), even though they contained the 4B6 epitope DTR. These results suggested that the epitopes of BC2, HMPV and 4B6 are APDTR, and that the epitope of OM1 could be DTRPA.

#### Reaction of mAbs with 120 substituted peptides

To determine whether some of these epitopes were indeed identical, substitutions were made and antibody binding examined. The SAPDTR sequence was altered by substituting each residue with any of the other 19 amino acids and the mAbs were tested using 120 (6 × 20)

6-mer peptides synthesised on the pins (Fig. 5). The results of BC2 were shown previously (Xing *et al.*, 1991). Like BC2, amino acids P and R are essential for HMPV binding to the peptide (Fig. 5); S can be completely substituted by any amino acid. Alanine (A) could be replaced by I, P, S, T, V and D, by L and N; T could be replaced by many amino acids. The reactions of HMPV are similar to BC2, except that more substitutions were allowable for A and D in SAPDTR (Fig. 5) (Xing *et al.*, 1991).

For 4B6, whose epitope is DTR (Fig. 2C), the adjacent amino acid P could be substituted by A and G (Fig. 6). D and R were not replaceable; T could be replaced by almost any other amino acid. These results indicate that PDTR could well be the real epitope of 4B6 as P is involved in the combining site of the mAb 4B6 with the

Table 2. Comparison of epitopes determined using different methods

|      | Common amino acids on pins | Inhibition                     | BSA-peptides | Substitution |
|------|----------------------------|--------------------------------|--------------|--------------|
| BC2  | PDTR                       | APDTR, PDTR(P)(A) <sup>a</sup> | APDTR        | APDTR        |
| HMPV | PDTR                       | APDTR, PDTR(P)(A) <sup>a</sup> | APDTR        | APDTR        |
| 4B6  | DTR                        | ?                              | APDTR        | PDTR         |
| OM1  | DTRP                       | ?                              | DTRPA        | ?            |

<sup>a</sup>The bracket signifies variation of epitopes.<sup>b</sup>Result is not clear.

peptide. For comparison, the same pins were also used to test mAb OM1 although they did not contain the intact epitope DTRPA of mAb OM1 (Fig. 7). The average absorbance value (0.39) in the reaction of SAPDTR peptides with OM1 was lower than that of BC2 (absorbance = 1.9), but it was also taken as 100% to compare with the reaction of other peptides. The results showed that D, T and R are not replaceable. The absorbance value (up to 2.357) clearly showed that some substituted peptides SFPDTR, SIPDTR, SVPDTR, SAADTR, SACDTR, SAIDTR, SALDTR and SAVDTR reacted strongly with mAb OM1, suggesting some amino acids at the *N*-terminal of these peptides may affect the conformation to favour binding of OM1 (Fig. 7).

#### Reaction of mAbs with amino acid peptides containing multiple substitutions

As demonstrated, with most of the antibodies the alteration of a single amino acid (such as P or R) could substantially alter antibody activity while alterations in A, D or T had little effect. It was of interest to determine whether multiple substitutions in A, D or T (while leaving P and R intact) could affect antibody binding. To do this a series of 29 peptides were designed with up to

four amino acids replaced in the peptide SAPDTR. It was shown that peptide could be made which appeared to have no relationship to the original peptide and yet still give good binding activity (Fig. 8). For example, the peptide VSPNQR (which retains only P and R of SAPDTR) gave the same reaction as APDTR with antibody BC2. By contrast, peptides CPPDIR and ASPDER, also retaining P and R, had no reactivity with BC2. While isolated peptides could be examined and discussed, no real pattern appeared, other than to state that P and R are crucial parts of the epitope, and surrounding amino acids, depending on what they are, have a major influence on the binding of antibody.

A second point of interest from this, was to compare the reactions of the four antibodies BC2, HMPV, 4B6 and OM1. Of all the data presented thus far it appeared that APDTR, PDTR or DTRPA (depending on the technique used) were the epitopes reacting with these antibodies. However, when the multiple substituted peptides were examined it was clear that the antibodies had different reactions. For example, peptide VSPNQR was reactive with BC2, but not with HMPV. By contrast, ASPDER and NSPDQR were non-reactive with BC2 but reactive with HMPV. Clearly, BC2 and HMPV antibodies are different and differences were also found on comparing the reactions of 4B6 with these. This could be a useful method of separating the reactions of antibodies which were presumed to be the same. It was noted that none of these substituted peptides reacted with OM1.

Table 3. Reaction of mAbs with BSA-peptide conjugates by ELISA<sup>a</sup>

| BSA-peptides   | BC2            | HMP | 4B6 | OM1 | 5C1 |
|----------------|----------------|-----|-----|-----|-----|
| 1. BSA-PDTR    | - <sup>b</sup> | -   | -   | -   | -   |
| 2. BSA-PDTRP   | -              | -   | -   | -   | -   |
| 3. BSA-PDTRPA  | -              | -   | -   | -   | -   |
| 4. BSA-APDTR   | +++            | ++  | ++  | ++  | -   |
| 5. BSA-DTRP    | -              | -   | -   | +   | -   |
| 6. BSA-DTRPA   | -              | -   | -   | ++  | -   |
| 7. BSA-P1-15   | -              | -   | -   | ++  | -   |
| 8. BSA-A-P1-15 | +++            | ++  | +++ | +++ | -   |
| 9. BSA-P1-24   | +++            | ++  | +++ | ++  | -   |
| 10. BSA-P5-20  | -              | -   | -   | -   | -   |
| 11. BSA-T4N1   | -              | -   | -   | -   | -   |

<sup>a</sup>The peptide was conjugated to BSA by glutaraldehyde method (Xing *et al.*, 1990), and coated to PVC plates, and tested by mAbs in direct binding ELISA.

<sup>b</sup>Absorbance value at 405 nm: (-) ≤ 0.25; 0.25 < (+) ≤ 1.0; 1.0 < (++) ≤ 1.5; 1.5 < (+++) at a mAb concn of 3.1 µg/ml.

## DISCUSSION

It has previously been shown that the 20 amino acid repeat unit (VNTR) within the mammary mucin (MUC1) is highly immunogenic—indeed many antibodies produced react with this repeat sequence and have been used in a  $\lambda$ gt11 expression system to isolate cDNA clones (Gendler *et al.*, 1987; Siddiqui *et al.*, 1988). In addition mAbs have been produced by immunizing with this synthetic peptide. Within this 20 amino acid sequence there is a major hydrophilic region and therefore presumably the most immunogenic, which consists of the amino acids SAPDTRPAP (Xing *et al.*, 1991). This study shows that four different anti-mucin antibodies react with a slightly different epitope in this sequence SAPDTRPAP. For example antibodies HMPV



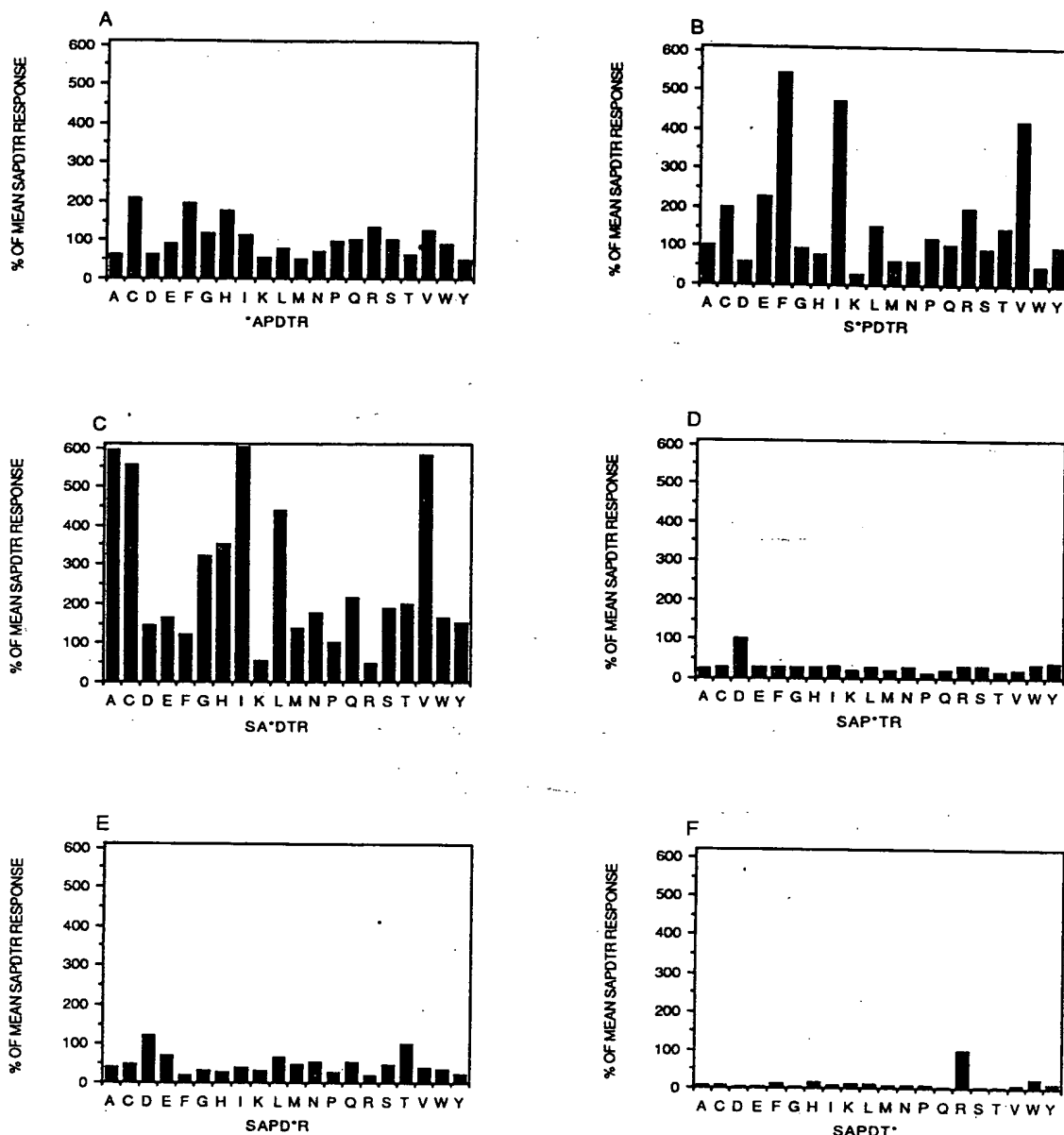


Fig. 7. Replacement set analysis of SAPDTR recognized by mAb OM1 (see legend Fig. 6 for further detail).

and BC2 react with (A)PDTR; 4B6 reacts with (P)DTR and OM1 reacts with DTRP(A) (the bracket signifies that different methods found different epitopes). It is suggested that within this region of nine amino acids there are several different epitopes of varying sizes from 3 amino acids (DTR) up to 5 amino acids such as APDTR and DTRPA recognized by these 4 mAbs, and some amino acids appear to be more critically involved with antibody binding than others. Clearly, P and R are the major determinants of activity—which is not surprising in view of their known role in conformation (P) or charge (R). However, in some cases (Fig. 6) multiple substitutions could be made and the antibody binding activity could be retained, for example a peptide of structure X-X-P-X-X-R (where X = almost any other amino acid) could react with some of the antibodies.

Clearly, the amino acids P and R may contribute directly to the linear epitope of the peptide, or it may be involved in such a conformation affecting the secondary and tertiary structure. In addition, the amino acids S and T in SAPDTR could be O-glycosylated—a likely possibility in view of the heavy glycosylation of mucins, and this may also affect antibody binding. We have presented some indirect evidence for conformational effects (Xing *et al.*, 1990, 1991) but have no information on the effects of glycosylation, which is currently under examination.

From the technical point of view it was noted that minor differences occurred using peptides of various lengths (4-mer, 5-mer and 6-mers) synthesized on pins, conjugated to BSA for direct binding and for those used in liquid phase inhibition studies. Firstly, some IgM antibodies such as 4B6 and OM1 are poorly inhibited by

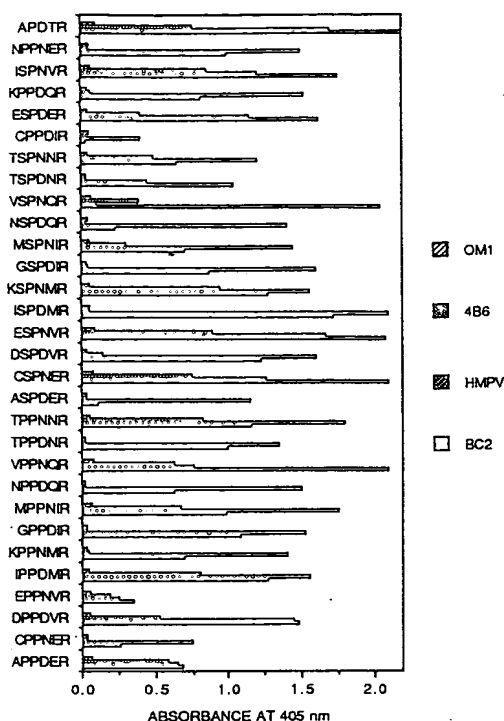


Fig. 8. Binding of mAbs BC2, HMPV, 4B6 and OM1 (coded on right side of Figure) to 29 6-mer synthetic peptides on the pins in an ELISA assay. Each peptide contains P and R, and 3-4 amino acids replaced at the position of S, A, D and T in parent peptide SAPDTR. Peptide APDTR was also synthesized and tested as a positive control.

short peptides. Secondly, different methods gave different results (Table 2). APDTR (not PDTR) is clearly the BC2 reactive epitope in inhibition studies (Xing *et al.*, 1990) and when the BSA-peptide conjugates were used in direct binding studies (Fig. 4). By contrast, using peptides synthesized on pins it appears that PDTR may be the BC2 reactive epitope (Fig. 2A). However, this is a simplified view since PDTR alone is non-reactive both in inhibition tests and in using pins (Fig. 2A, 3A), and when A is added to the C-terminal of the 4-mer PDTR, the peptide reacts (Figs 2A, 3A). Using BSA-peptide conjugates we demonstrated that BC2 and HMPV only reacted with BSA-conjugates which contained the APDTR sequence. By contrast 4B6 was able to react with PDTR on the pins, but not with PDTR conjugated to BSA by glutaraldehyde method. It was also found that the mAb OM1 bound to BSA-DTRPA but not to BSA-PDTRPA. In addition, mAb 4B6 did not react with the peptide p1-15, nor with the conjugates of short peptides and BSA, such as BSA-PDTR, BSA-DTRPA, BSA-DTRP, BSA-PDTRP and BSA-PDTRPA, although they are DTR-containing peptides. These results are difficult to explain but indicate that peptides bound to pins and to BSA (by glutaraldehyde) may have a different conformational structure. Similar results have also been reported elsewhere (Price *et al.*, 1990) in that mAb NCRC 11 did not bind BSA-p1-20 containing the reactive RPAP epitope, and it was suggested that these particular peptides could lack the appropriate

secondary or tertiary structure (Price *et al.*, 1990; Tendler, 1990).

It was observed that flanking amino acids at the N- or C-terminal of peptides could affect the reaction of mAbs. For example, the epitopes of BC2 and HMPV appeared to be APDTR, but peptides PDTRPA, PDTRP both inhibited BC2 and HMPV binding to HMFG (Fig. 3, Table 2). Similar results were found in the reaction of OM1 with peptides SFPDTR, SIPDTR, SAADTR, SAIDTR and SAVDTR, although these peptides contain only 3 amino acids (DTR) of the minimum epitope DTRPA (Fig. 7). These results suggest that the epitope may consist of two parts: one is crucial (where the amino acid cannot be substituted, such as P and R in the BC2 epitope APDTR); and the other, being the variable part where the reaction with mAbs varies according to the length and content of amino acids in the peptide.

A further point to note is to question the specificity of the reactions observed, such as, the epitope of BC2 and HMPV appears to be APDTR. However, from earlier studies with single amino acid substitutions the critical amino acids in this epitope are P and R since there are many other amino acids that can be substituted for A, D or T and the antibodies BC2 and HMPV would still react (Xing *et al.*, 1991) (Fig. 5). It is possible that many of the reactions observed with the antibody, for example, the reaction with salivary gland, kidney, stomach and the like, may indeed not be due to the APDTR but to another peptide, all of which contain P and R and other amino acids. This will take some time to determine but the recent cloning of the same cDNA from pancreatic tissue as from breast cancer, indicates that the reaction with pancreas is probably due to APDTR and not to some unrelated peptide (Lan *et al.*, 1990).

The antibody OM1 made to an ovarian cancer cell line was found to react with both HMFG, and synthetic peptides derived from breast cancer mucin of MUC1 origin (de Kretser, 1988; Layton *et al.*, 1990) and its epitope is now defined as DTRPA. This clearly demonstrates that ovarian cancer has the same or a similar protein core as breast cancer and show that "mammary" mucins exist not only in breast cancer, but also in ovarian cancer. Indeed, the same mucin was also detected in lung and pancreatic cancer (Xing *et al.*, 1989b; Lan *et al.*, 1990). It will be of interest to determine whether other unique mucins are found in ovary, pancreas and lung as several unique mucins have recently been found in the gastrointestinal and tracheo-bronchial tracts (Gum *et al.*, 1989, 1990; Prochet *et al.*, 1991).

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